bio-protocol Preprint

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Isolation and characterization of peritoneal immune cells

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An abbreviated version of this protocol was published in Science in Oct 2021

p21 produces a bioactive secretome that places stressed cells under immunosurveillance

DOI: 10.1126/science.abb3420

Detailed protocol

Isolation of peritoneal immune cells

See also:

- Ray A, Dittel BN. Isolation of mouse peritoneal cavity cells. JoVe 2010 PMID 20110936
- Layoun, A., Samba, M., Santos, M. M. Isolation of murine peritoneal macrophages to carry out gene expression analysis upon Toll-like receptors stimulation. JoVe 2015 PMID 25993651

Preparation:

- Ice cold DPBS (chilled at 4C) sterile
- 10 ml syringes
- 20G needles
- Dissection tools
- 50 ml falcons
- Cooling centrifuge for 50 ml falcons
- For culture: DMEM or DMEM/F12 with 10% FBS, antibiotics works well (otherwise, RPMI 1640 is recommended in literature)
- Optional: eliciting agent, Brewer's medium Thioglycollate, 3.8%, autoclaved

Protocol:

- Euthanize mouse by CO2
- Open outer skin layer of belly without damaging the peritoneal membrane (important! Do not make ANY holes!), free up the whole belly/ peritoneal cavity to improve visualization
- Fill a 10 ml syringe with ice cold PBS, attach a 20G needle
- Central, slightly right (on side of spleen) lift the peritoneal skin with blunt forceps, insert the needle for a few millimeters without damaging/poking any of the internal organs (important! If you damage any organs discard the mouse!)
- Eject 10 ml of PBS slowly into the peritoneal cavity, the belly will swell a lot but 10 ml will fit well (if there are any holes in the peritoneal membrane, the liquid will leak out and the mouse will not be useful)
- While leaving the needle inside, massage/tap the sides of the belly back and forth to loosen any attached cells from the organs (~10 times)
- Aspirate PBS slowly. Often fat tissue or pancreas will get caught in the needle, if this happens move the needle or pull the needle out so that the tissue will close the hole and insert the needle at a different location
- Recovery of PBS should be at least 8.5 ml (average 9 ml)
- Remove 20 G needle (securely!)
- Dispense suspension into prechilled 50 ml falcon
- Spin falcon at 400 g, 10 min, 4 C cooling centrifuge
- Remove supernatant and resuspend pellet in ~300 ul medium or PBS
- Count with hemocytometer and use for experiments

Yield, average 1 million cells per mouse
B-cells (~50%), Macrophages (~40%), T-cells (~10%)

To increase yield, mice can be injected with Thioglycollate 3-5 days prior harvest, this will increase cell number 5-10 fold (but cells have different properties and the distribution of cell populations is quite different)

Flow cytometry stainings;

- Cd11b
- TCRbeta
- B220

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Li, H. , Deursen, J. and Baker, D. (2022). Isolation and characterization of peritoneal immune cells. Bio-protocol Preprint. bio-protocol.org/prep1823.
2. Sturmlechner, I., Zhang, C., Sine, C. C., Deursen, E. V., Jeganathan, K. B., Hamada, N., Grasic, J., Friedman, D., Stutchman, J. T., Can, I., Hamada, M., Lim, D. Y., Lee, J., Ordog, T., Laberge, R., Shapiro, V., Baker, D. J., Li, H. and Deursen, J. M. V.(2021). p21 produces a bioactive secretome that places stressed cells under immunosurveillance. Science 374(6567). DOI: 10.1126/science.abb3420